PCT/AU00/01182

AU 00/1182

4

5/4

#2

Patent Office
Canberra

10/089273

I, LISA TREVERROW, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 3057 for a patent by MONASH UNIVERSITY filed on 24 September 1999.

WITNESS my hand this
Twenty-fifth day of October 2000

Lisa Treverrow

LISA TREVERROW
TEAM LEADER EXAMINATION
SUPPORT AND SALES

Regulation 3.2

Monash University

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Peptides"

The invention is described in the following statement:

PEPTIDES

FIELD OF THE INVENTION

The present invention relates to human T cell epitopes of the latex allergen Hev b 5, to nucleic acids encoding them, to related vectors and host cells, to therapeutic compositions, methods of treatment and sensitivity detection involving them.

BACKGROUND OF THE INVENTION

IgE mediated hypersensitivity to latex has emerged as a serious occupational health problem since the introduction of universal precautions in the mid 1980s (1, 2). The use of latex gloves for barrier protection may lead to sensitization, especially of health care workers (HCW), to protein allergens present in the natural rubber latex. The prevalence of latex sensitization amongst HCW has been reported from 8.2-22% (3-7). Allergic reactions to latex range from urticaria, rhinoconjunctivitis, asthma (8), and angioedema to severe generalised anaphylaxis in some cases (9). Since the only form of treatment available at present is allergen avoidance and symptomatic relief, there is an urgent need for the development of specific immunotherapy for this condition. Thus, the immunological characterisation of latex allergens is an important breakthrough in the development of rational curative treatments for latex allergy.

Several allergens from the rubber plant *Hevea brasiliensis* have been identified (10-18). Based on IgE binding studies, certain latex allergens seem to be preferentially recognised by particular risk groups (Hev b 1 and 3 by children with spina bifida and Hev b 5 and 6.02 by HCW (19)). Hev b 5, a highly acidic and proline rich protein with a predicted predominantly random secondary structure, has been shown to react with IgE from 92% of HCW and 56% of spina bifida patients with latex allergy (16). Thus Hev b 5 has been identified as a major allergen in natural rubber latex. Interestingly, the amino acid sequence of Hev b 5 shows 46% identity to another acidic protein identified in kiwi fruit (*Actinidia deliciosa*) (16) and

this may provide a molecular explanation for the high frequency of fruit hypersensitivity seen in latex allergic patients (20).

The production of allergen specific IgE by B cells and release of inflammatory mediators by mast cells and eosinophils result in the effector response of allergic disease. However, it is well established that these events are orchestrated by allergen-specific $CD4^+$ T cells with a Th_2-type cytokine profile. T cell reactive determinants have been reported for another major latex allergen, Hev b 1 (21), but not for Hev b 5. Thus, characterisation of the T cell response to Hev b 5 is critical in the development of specific immunotherapy.

Murine T cell epitopes of Hev b 5 have been identified previously in BALB/c mice. Although there is precedent in the literature for shared murine and human allergenic T cell epitopes (31), this is certainly not the normal situation and was not expected by the present inventors. It is interesting to note that the dominant murine T cell determinants were Hev b 5 (1-20), (37-56), (73-92), (82-101), (109-128), (118-137) and (127-146) whereas the immunodominant human T cell determinant identified by the present inventors which was recognised by all five subjects with IgE reactivity to rHevb5/MBP was Hev b 5 (46-65), which was only a minor murine T cell determinant. This variation between murine and human immunodominance demonstrates the difficulties associated with predicting immune response between species, and highlights the surprising results achieved by the present inventors.

It is an object of the present invention to identify human T cell epitopes of the latex antigen Hev b 5 which may be useful in detection of sensitivity to Hev b 5 and treatment of human latex allergy. Other objects of the invention will become apparent from the following detailed description thereof.

SUMMARY OF THE INVENTION

According to one embodiment of the present invention there is provided an isolated human T cell epitope of Hev b 5 or a homologue thereof.

According to another embodiment of the present invention there is provided an isolated T cell epitope of Hev b 5 having an amino acid sequence as shown in Figure 1, or a homologue thereof.

According to a further embodiment of the present invention there is provided an isolated T cell epitope of Hev b 5 having amino acid sequence selected from that of

Hev b 5 (37-56)
Hev b 5 (46-65)
Hev b 5 (55-74)
Hev b 5 (109-128)
Hev b 5 (118-137)

as shown in Figure 1, or a homologue thereof.

According to another embodiment of the invention there is provided an isolated T cell epitope of Hev b 5 having amino acid sequence of Hev b 5 (46-65) as shown in Figure 1.

According to a further embodiment of the invention there is provided an isolated peptide comprising at least one T cell epitope of Hev b 5 or a homologue thereof, as defined above.

The present invention also relates to isolated nucleic acids encoding T cell epitopes of Hev b 5 or homologues thereof or isolated peptides comprising a T cell epitope of Hev b 5 or a homologue thereof as well as functional equivalents of such nucleic acids.

Monoclonal antibodies which react specifically with isolated T cell epitopes or isolated peptides also form an aspect of the invention as do expression vectors comprising nucleic acids as outlined above, host cells including such expression vectors and the isolated peptides produced by these host cells.

Therapeutic compositions comprising isolated T cell epitopes of Hev b 5 or their homologues or isolated peptides or their homologues as outlined above, in association with a pharmaceutically acceptable carrier or diluent, additionally form an aspect of the invention.

A further embodiment of the invention relates to a method of treating sensitivity of Hev b 5 or sensitivity to an allergen which is immunologically cross-reactive to Hev b 5 in a patient in need of such treatment which comprises administering to the patient a therapeutically effective amount of a therapeutic composition as outlined above. A similar treatment method is effective for treatment of latex allergy.

According to a further embodiment of the invention there is provided use of an isolated T cell epitope or a homologue thereof as outlined above or an isolated peptide or homologue thereof as also outlined above in preparation of a therapeutic composition for treatment of sensitivity to Hev b 5 or sensitivity to an allergen which is cross-reactive to Hev b 5.

The present invention also includes use of an isolated T cell epitope of Hev b 5 or a homologue thereof as outlined above or an isolated peptide or homologue thereof as also outlined above in preparation of a therapeutic composition for treatment of latex allergy.

The present invention additionally relates to a method for detecting sensitivity to Hev b 5 in an individual, comprising combining a blood sample obtained from the individual with an isolated T cell epitope of Hev b 5 or a homologue thereof, as outlined above, or an isolated peptide or homologue thereof, as also outlined above, under conditions appropriate for binding of blood components with the peptide, and determining the extent to which such binding occurs as indicative of sensitivity of the individual to Hev b 5.

BRIEF DESCRIPTION OF THE FIGURES

The present invention will be described further with reference to the Figures, a description of which follows:

Figure 1. Primary amino acid sequences of overlapping synthetic peptides, spanning the entire length of the Hev b 5 molecule. Amino acid residues are indicated with the single letter code. All peptides are 20 mers with 11 amino acid overlap except for the last two peptides that overlap by 15 residues.

Figure 2. Dot immunoblot analysis of serum IgE reactivity with latex allergens for latex allergic patients 1-6.

Figure 3. Proliferation of (A) PBMC and (B) latex specific T cell line of patient 2 in response to latex allergens

(A) PBMC proliferative response to LAL (0.03-100 μg/ml) rHevb5/MBP (5-40 μg/ml), MBP (5-40 μg/ml) was assessed by ^{3}H-thymidine incorporation. Tetanus Toxoid (tet-tox) (0.3 Lf/ml) and Lymphocult-T (10 IU/ml) were included as controls. Results are expressed as cpm $\pm$ SD of triplicate cultures. Cells alone in the absence of antigen were included to assess background levels of cell proliferation.

(B) T cell line proliferation in the presence of Hev b 5 peptides (10,30 μg/ml), LAL (3-100 μg/ml), rHev b5/MBP (10 μg/ml), MBP (10 μg/ml), PHA (3 μg/ml) and Lymphocult-T (10 IU/ml) was assessed by ^{3}H-thymidine incorporation. T+APC alone, T alone and APC alone were included to assess background levels of cell proliferation.

Figure 4. (A) Proliferation and (B) IL-5 production by latex specific T cell line of latex allergic patient 4.

(A) Proliferation of T cells in response to Hev b 5 peptides (30, 10 μg/ml), LAL (100, 0, 10, 3 μg/ml) and PHA (3 μg/ml) assessed by ^{3}H-thymidine incorporation (values shown are averages of triplicate samples with SD indicated). Background level of cell proliferation (T+APC) is indicated by the horizontal line at 1000 cpm.

(B) Production of IL-5 in the culture supernatants determined by ELISA. Only the indicated peptides were tested for their ability to stimulate T cell production of IL-5. NT indicates peptides that were not tested in the IL-5 assay. Values indicate averages of duplicate samples.

Figure 5. Comparison of human and murine T cell determinants of Hev b 5.

Latex allergic patient (1-5) T cell stimulation indices $\geq$ 2.5 in response to Hev b 5 peptide stimulation are shown by the hatched boxes. Previously identified murine T cell epitopes (30) are shown in shading.

DETAILED DESCRIPTION OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The present invention relates to isolated T cell epitopes of Hev b 5 and to homologues thereof. Amino acid sequences of T cell epitopes of Hev b 5 which have been identified by the present inventors are provided in Fig 1. By the term "isolated" it is intended to mean that

peptides or nucleic acids according to the invention are substantially free of cellular materials, culture medium, chemical precursors or byproducts which may have been involved in synthesis or production or may have been present in the environment from which the peptide or nucleic acid was obtained. There has therefore been some intervention to separate and purify at least to some extent the peptides or nucleic acids from other biological materials or chemical species.

As used herein the term "homologue" includes within its scope fragments and modified forms of the peptide concerned which may result from single or multiple amino acid deletions, insertions or translocations, but which continue to exhibit proliferative responses to latex specific T cell lines from latex allergic patients (T cell stimulation indices) in excess of about 3.0 and preferably in excess of about 5.0. T cell proliferative response can readily be tested by the methods outlined in the experimental section which follows. Fragments will preferably include at least 5 amino acids, particularly preferably at least 10 amino acids.

Also included within the present invention are peptides that comprise at least one T cell epitope of Hev b 5 or a homologue thereof in conjunction with other amino acids (which may or may not be naturally occurring as amino acid analogues) or other chemical species. In a preferred aspect of the invention such peptides may comprise at least two T cell epitopes of Hev b 5. Such peptides with at least two T cell epitopes of Hev b 5 are desirable for increased therapeutic effectiveness.

It is possible to modify the structure of a peptide according to the invention for various purposes such as for increasing solubility, enhancing therapeutic or preventative efficacy, enhancing stability or increasing resistance to proteolytic degradation. A modified peptide may be produced in which the amino acid sequence has been altered, such as by amino acid substitution, deletion or addition, to modify immunogenicity and/or reduce allergenicity. Similarly components may be added to peptides of the invention to produce the same result.

For example, a peptide can be modified so that it maintains the ability to induce T cell anergy and bind MHC proteins without the ability to induce a strong proliferative response or possibly, any proliferative response when administered in immunogenic form. In this instance, critical binding residues for the T cell receptor can be determined using known techniques (for example substitution of each residue and determination of the presence or absence of T cell reactivity). Those residues shown to be essential to interact with the T cell receptor can be modified by replacing the essential amino acid with another, preferably similar amino acid residue (a conservative substitution) whose presence is shown to enhance, diminish but not eliminate, or not effect T cell reactivity. In addition, those amino acid residues which are not essential for T cell receptor interaction can be modified by being replaced by another amino acid whose incorporation may enhance, diminish or not effect T cell reactivity but does not eliminate binding to relevant MHC proteins.

Modifications may involve replacing an amino acid shown to be essential to interact with the MHC protein complex with another, preferably similar amino acid residue (conservative substitution) whose presence is shown to enhance, diminish but not eliminate, or not effect T cell activity. In addition, amino acid residues which are not essential for interaction with the MHC protein complex but which still bind the MHC protein complex can be modified by being replaced with another amino acid whose incorporation may enhance, not effect, or diminish but not eliminate T cell reactivity. Preferred amino acid substitutions for non-essential amino acids include, but are not limited to substitutions with alanine, glutamic acid, or a methyl amino acid.

Peptides of the invention may also be modified to incorporate one or more polymorphisms resulting from natural allelic variation and D-amino acids, non-natural amino acids or amino acid analogues may be substituted into the peptides to produce modified peptides which fall within the scope of the invention. Peptides may also be modified by conjugation with polyethylene glycol (PEG) by known techniques. Reporter groups may also be added to facilitate purification and potentially increase solubility of the peptides according to the invention. Other well known types of modification including insertion of specific

endoprotease cleavage sites, addition of functional groups or replacement of hydrophobic residues with less hydrophobic residues as well as site-directed mutagenesis of DNA encoding the peptides of the invention may also be used to introduce modifications which could be useful for a wide range of purposes. The various modifications to peptides according to the invention which have been mentioned above are mentioned by way of example only and are merely intended to be indicative of the broad range of modifications which can be effected.

The T cell epitopes of Hev b 5 and isolated peptides comprising them can be produced either by synthetic chemical methods or by recombinant DNA techniques in a host cell transformed with a nucleic acid having a sequence encoding the peptide concerned. Nucleic acids encoding T cell epitopes of Hev b 5 or their homologues and nucleic acids encoding peptides which comprise T cell epitopes of Hev b 5 or their homologues also form a part of the invention. Such nucleic acids can be as cDNAs which encode the relevant peptide sequence and can be produced chemically or mechanically, by known techniques. As utilised throughout this specification, the term "functional equivalent" of a nucleic acid encompasses nucleic acids which are capable of hybridising to a complimentary oligonucleotide encoding a T cell epitope of Hev b 5 or their homologues or a peptide comprising a T cell epitope of Hev b 5, or a nucleic acid which encodes for a functional equivalent or homologue of a T cell epitope of Hev b 5 or peptides comprising it.

Such nucleic acids may be useful for recombinant production of T cell epitopes of Hev b 5 or proteins comprising them by insertion into an appropriate vector and transfection into a suitable cell line. Such expression vectors and host cell lines also form an aspect of the invention.

In producing peptides by recombinant techniques, host cells transformed with a nucleic acid having a sequence encoding a peptide according to the invention or a functional equivalent of the nucleic acid sequence are cultured in a medium suitable for the particular cells concerned. Peptides can then be purified from cell culture medium, the host cells or both using techniques well known in the art such as ion exchange chromatography, gel filtration

chromatography, ultrafiltration, electrophoresis or immunopurification with antibodies specific for the peptide.

Nucleic acids encoding for Hev b 5 or homologues thereof or peptides comprising Hev b 5 or homologues thereof may be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells such as Chinese hamster ovary cells (CHO). Suitable expression vectors, promoters, enhancers and other expression control elements are referred to in Sambruck *et al* (34). Other suitable expression vectors, promoters, enhancers and other expression elements are well known to those skilled in the art. Examples of suitable expression vectors in yeast include Yep Sec 1 (35); pMFa (36); JRY88 (37) and pYES2 (Invitrogen Corporation, San Diego, CA). These vectors are freely available as are baculovirus and mammalian expression systems. For example, a baculovirus system is commercially available (ParMingen, San Diego, CA) for expression in insect cells while the pMsg vector is commercially available (Pharmacia, Piscataway, NJ) for expression in mammalian cells.

For expression in *E. coli* suitable expression vectors include among others, pTrc (38) pGex (Amrad Corporation, Melbourne, Australia); pMal (N.E. Biolabs, Beverley, MA); pRit5 (Pharmacia, , Piscataway, NJ); pEt-11d (Novagen, Maddison, WI) (39) and pSem (40). The use of pTRC, and pEt-11d, for example, will lead to the expression of unfused protein. The use of pMal, pRit5, pSem and pGex will lead to the expression of allergen fused to maltose E binding protein (pMal), protein A (pRit5), truncated β-galactosidase (PSEM) or glutathione S-transferase (pGex). When a T cell epitope of Hev b 5 or a homologue thereof or a peptide comprising it is expressed as a fusion protein, it is particularly advantageous to introduce an enzymatic cleavage site at the fusion junction between the carrier protein and the peptide concerned. The peptide of the invention may then be recovered from the fusion protein through enzymatic cleavage at the enzymatic site and biochemical purification using conventional techniques for purification of proteins and peptides. Examples of enzymatic cleavage sites include those for blood clotting factor Xa or thrombin for which the appropriate enzymes and protocols for cleavage are commercially available. The different vectors also have different promoter regions allowing constitutive or inducible expression or temperature

induction. It may additionally be appropriate to express recombinant peptides in different *E. coli* hosts that have an altered capacity to degrade recombinantly expressed proteins. Alternatively, it may be advantageous to alter the nucleic acid sequence to use codons preferentially utilised by *E. coli*, where such nucleic acid alteration would not effect the amino acid sequence of the expressed proteins.

Host cells can be transformed to express the nucleic acids of the invention using conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection or electroporation. Suitable methods for transforming the host cells may be found in Sambruck *et al* (34), and other laboratory texts. The nucleic acid sequence of the invention may also be chemically synthesised using standard techniques.

In addition to recombinant production of peptides according to the invention, the nucleic acids may be utilised as probes for experimental or purification purposes.

Another aspect of the present invention relates to antibodies which are specifically reactive to isolated T cell epitopes or homologues thereof or peptides including isolated T cell epitopes or homologues according to the invention. Such antibodies may be used to standardise allergen extracts or to isolate naturally occurring Hev b 5 peptides. Methods of production of antibodies are well known in the art.

Peptides or antibodies according to the present invention may also be used for detecting and diagnosing latex allergy or more specifically Hev b 5 sensitivity or sensitivity to allergens which are immunologically cross-reactive to Hev b 5. Such detection and diagnosis techniques may involve the combining of blood or blood products obtained from an individual to be assessed with T cell epitopes of Hev b 5 or homologues thereof or peptides comprising such epitopes, under conditions appropriate for binding of components in the blood (for example antibodies, T-cells, B-cells) with the peptide concerned, and the subsequent determination of the extent of such binding as indicative of Hev b 5 sensitisation.

T cell epitopes of Hev b 5 or homologues thereof or peptides comprising such epitopes or homologues may be administered to a latex allergic individual, or an individual allergic to an antigen cross-reactive with Hev b 5, in order to modify the allergic response of the individual. Preferably such treatment regimes are capable of modifying the B-cell response, the T-cell response or both the B-cell and T-cell response of the individual concerned. As used herein modification of the allergic response of the individual suffering from latex allergy or sensitive to Hev b 5 or a cross-reactive allergen can be defined as non-responsiveness or diminution in symptoms to the allergen, as determined by standard clinical procedures (41). Diminution in the symptoms includes any reduction in allergic response of an individual to the allergen after a treatment regimen has been completed. This diminution may be subjective or clinically determined, for example by using standard skin tests known in the art. Peptides according to the present invention which comprise at least one T cell epitope are particularly desirable for therapeutic purposes. Preferably however, the therapeutic peptides will comprise at least two Hev b 5 T cell epitope. Reference to an epitope indicates the basic element or smallest unit of recognition by a receptor, particularly immunoglobulins, histocompatibility antigens and T cell receptors, where the epitope comprises amino acids essential to receptor recognition.

Exposure of the latex allergic patients to peptides according to the invention which comprise at least one T cell epitope or a homologue thereof may tollerize or anergize appropriate T cell sub-populations such that they become unresponsive to the protein allergen and do not participate in stimulating an immune response upon such exposure. Preferably the peptides according to the invention will retain immunodominant T cell epitopes but possess abrogated IgE binding.

Administration of a peptide of the invention may modify the lymphokine secretion profile as compared with exposure to naturally occurring Hev b 5 allergen. This exposure may also influence T cell sub-populations which normally participate in the allergic response to migrate away from the site or sites of normal exposure to the allergen and towards the site or sites of therapeutic administration. This redistribution of T cell sub-populations may ameliorate

or reduce the ability of an individual's immune system to stimulate the usual immune response at the site of normal exposure to the allergen, resulting in a diminution of the allergic symptoms.

Peptides according to the present invention may be provided in the form of diagnostic compositions which may be used in diagnostic tests for allergy such as radio-allergosorbent test (RAST), paper radioimmunosorbent test (PRIST), enzyme linked immunosorbent assay (ELISA), radioimmuno assay (RIA), immunoradiometric assay (IRMA), luminescence immuno assay (LIA), hystermine release assays and IgE immunoblots.

Therapeutic compositions according to the present invention may include T cell epitopes of Hev b 5, their homologues or proteins comprising the T cell epitopes or homologues in association with a pharmaceutically acceptable carrier or diluent. Examples of pharmaceutically acceptable carriers and diluents are discussed in detail in Remington's Pharmaceutical Sciences (42), the disclosure of which is included herein in its entirety by way of reference.

As used herein "pharmaceutically acceptable carrier or diluent" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like, as are well known in the art. It is additionally possible for peptides according to the invention to be administered in association with supplementary active compounds. Naturally, the peptides of the invention must be compatible with carriers, diluents or supplementary active compounds which they may be administered in conjunction with.

Administration of the therapeutic compositions can be carried out using known procedures at dosages and for periods of time effective to reduce sensitivity (ie. reduce the allergic response) of the individual to the allergen. Effective amounts of the therapeutic compositions will vary according to factors such as the degree of sensitivity of the individual to latex, the age, sex, weight and general well being of the individual concerned.

The peptides of the invention may be administered in a convenient manner such as by injection, by oral administration, inhalation, transdermal application or as a suppository. Depending upon the route of administration, the active compound may need to be coated with a material to protect it from the action of enzymes (enteric coating), acids and other natural conditions which could cause its inactivation. For example, the peptides of the invention may be protected by use of an inert diluent or an assimable edible carrier, they may be enclosed in a hard or soft shell gelatine capsule, compressed into tablets, or incorporated directly into the individual's diet. The peptides may be administered in association with liposomes.

By way of example an amount of between about 1 microgram and about 3 milligrams and preferably from about 20 micrograms to about 500 micrograms of a T cell epitope of Hev b 5 or a homologue thereof or a peptide comprising such T cell epitope or homologue may be administered to a patient, most preferably by way of injection. The dosage regimen may be adjusted to provide the optimum therapeutic response and for example several divided doses may be administered daily, weekly, monthly, quarterly or yearly as needed, or the dose may be proportionately reduced or increased as indicated in the particular therapeutic situation.

Further details of the present invention will be described by way of example only, in the following experimental section.

EXPERIMENTAL

Methods

Subjects

Latex allergic HCW were recruited from the Alfred Hospital Allergy Clinic (with informed consent following approval by Alfred Hospital Ethics Committee). All subjects had severe

clinical symptoms of IgE mediated latex hypersensitivity and a grade 3-4/4 score of latex-specific serum IgE (Table I) measured using the Kallestad Allercoat™ EAST system (Sanofi-Pasteur Diagnostics, USA). The latex EAST score in non-allergic individuals is 0/4 and <0.18 Allercoat™ EAST Units (AEU/ml).

Antigens

Low-ammoniated latex: Low ammoniated latex (LAL; Ansell, Australia) was centrifuged at 20,000 rpm for 20 minutes at room temperature. The middle clear layer was collected, dialysed against phosphate buffered saline (PBS) overnight at 4°C, filter sterilised and the protein concentration determined using the BCA protein assay kit (Pierce, USA).

Hev b 5: A construct comprising the Hev b 5 encoding cDNA in the pMAL/c-2 vector was generated as part of a maltose binding protein (MBP) fusion protein (rHev b 5/MBP) as described previously (16). The rHEV b 5/MBP was purified from the crude bacterial protein extract by affinity chromatography on an amylose column (New England Biolabs, USA) according to the manufacturer's instructions. As a control protein, MBP fused to the LacZα protein was produced by expressing the pMAL/c-2 vector alone as described previously (16).

Tetanus toxoid (TT) and *Phytohaemagglutinin (PHA)*: These control antigens were purchased from Sigma, USA.

Peptides: Synthetic peptides (20-mers overlapping by 11 or, for the two N-terminal peptides, 15 residues) spanning the entire length of the Hev b 5 molecule were purchased from Chiron Technologies (Clayton, Australia; Fig 1). The purity of the peptides, as determined by high-performance liquid chromatography, was greater than 90%. Peptides were dissolved in sterile PBS (Sigma, USA) to achieve a stock concentration of 1 mg/ml.

Immunoblotting

Patient serum IgE reactivity to latex allergens was analysed by dot immunoblots. LAL (25 μg), rHev b 5/MBP (7 μg) and MBP (4.5 μg) were applied side by side on a nitrocellulose membrane (Schleichner and Schuell, Germany) in a 5 μl volume and allowed to soak in. The amount of MBP was estimated to be equivalent to that in the rHev b 5/MBP aliquot. The membrane was then blocked, incubated in patient sera and IgE binding detected according to our established protocols (22). Briefly, the blots were blocked in 10% w/v skim milk powder in PBS and washed once in PBS containing 0.2% v/v Tween 20 and twice in PBS alone. Blots were then incubate din patients' sera (diluted 1:4 in PBS/0.5% BSA) overnight at room temperature with shaking before washing as described above. Finally, IgE antibody binding was detected by incubation in rabbit anti-human IgE (Dako, Denmark) followed by horse radish peroxidase-labelled anti-rabbit antibodies (Promega, USA), washing in between, and developing in the substrate 4-chloro-1-napthol (Sigma) after the final wash.

Generation of latex specific T cell lines

Latex specific T cell lines were isolated using our well established methods for the generation of allergen specific oligoclonal T cell populations (23). An initial primary culture was performed for each patient to determine the optimal concentration of LAL for stimulation. For this, peripheral blood mononuclear cells (PBMC) separated from heparinised venous blood by density centrifugation (2 x 10^5/well) were stimulated with LAL over a concentration range 0.03 to 100 μg/ml, with control cultures of PBMC alone or stimulated with tetanus toxoid. Gibco Life Technologies supplemented with 2 mmol/L L-glutamine, 100 IU/ml penicillin-streptomycin and 5% screened, heat inactivated human AB serum [Sigma]). Optimal responses were observed at 10 and 30 μg/ml LAL in all cases. Therefore, to generate T cell lines, PBMC (2 x 10^6/well) were stimulated for 1 week with LAL at a concentration of 20 μg/ml in 24-well tissue culture plates (Costar, USA). After 7 days, lymphoblasts (2 x 10^6/well) were restimulated for one week with LAL at a concentration of 20 μg/ml in the presence of an equal number of irradiated (3000 rad) autologous PBMC as antigen presenting cells (APC). On days 2 and 4, cultures were supplemented with Lymphocult-T (5% v/v; Biotest Folex, Germany) and fresh medium. At the end of the

second week, lymphoblasts were restimulated for one week with rHev b 5/MBP at a concentration of 20 μg/ml as described above. Oligoclonal T cells were recovered, washed and tested in proliferation and cytokine assays (as described below) either fresh or after cryopreservation in liquid nitrogen. We have previously shown that $CD4^+$ T cells are preferentially expanded in these cultures.

Oligoclonal T cell proliferation assays

T cells (5×10^5) were incubated in 96-well round bottom plates (Linbro, ICN Biomedicals, USA) in triplicate with equal numbers of irradiated APC in the presence of LAL at concentrations ranging from 0.03-100 μg/ml, rHev b 5/MBP (10 and 20 μg/ml), MBP (10 and 20 μg/ml), overlapping peptides of the Hev b 5 sequence (10 and 30 μg/ml), Lymphocult-T (10 IU/ml) or PHA (3 μg/ml). Cultures of T cells and APC in the absence of antigen, T cells alone and APC alone in the absence of antigen were included as controls. After 72 hours cultures were pulsed with 1 μCi of ^{3}H-thymidine (^{3}HTdR; Du Pont, NEN) and harvested 12-16 hours later. Proliferation as correlated with ^{3}HTdR incorporation was measured by liquid scintillation spectroscopy. Results are expressed as mean counts per minute (cpm) for triplicate cultures with standard deviation (SD; ≤ 20% for all experiments).

Mitogenicity and toxicity assays

Mitogenicity of latex antigens were excluded by testing the ability of LAL, rHev b 5/MBP and Hev b 5 peptides to stimulate a house dust mite specific three-week T cell line. No reactivity to latex allergens was observed (data not shown). Toxicity was assessed by coculturing the same antigens with house dust mite specific T cells in the presence of Lymphocult-T at 10 IU/ml. Latex antigens failed to inhibit the Lymphocult-T induced proliferation even at the highest concentration (data not shown) excluding toxicity.

Production of IL-5 and IFNγ by latex specific oligoclonal T cells

The secretion of IL-5 and IFNγ by oligoclonal latex specific T cells was determined by ELISA on culture supernatants. Representative stimulatory and non-stimulatory peptides were selected and T cells cultured in triplicate with these peptides. At 48 hours 50 μl of supernatant was harvested from the cultures and IL-5 and IFNγ were assayed by ELISA using paired cytokine antibodies (from Pharmingen Becton Dickinson, USA and Endogen, USA respectively). For this, opaque flat bottom ELISA plates (Nalgene, Nunc International) were coated with 30 μl capture antibody at 2 μg/ml in 0.1 M $NaHCO_3$ pH 8.2 coating buffer overnight at 4°C. After washing three times with PBS/0.5% Tween the wells were blocked with 3% BSA/PBS at room temperature for 2 hours. The plates were washed and 30 μl pooled triplicate culture supernatants added in duplicate. After incubation overnight at 4°C and washing, bound cytokine was revealed by incubation with 30 μl biotinylated detecting antibody (1 μg/ml for IL-5, 0.5 μg/ml for IFNγ) for 45 minutes at room temperature followed by streptavidin-biotinylated HRP (Amersham Pharmacia Biotech) at 1:2000 dilution for 30 minutes and enhanced chemiluminescence substrate (NEN Life Science). Light emission was read immediately on a Lumicount microplate Glow Luminometer (Packard Instrument Company). A standard curve was run each time using known concentrations of standard cytokine samples and the concentrations of IL-5 and IFNγ in the culture supernatants calculated accordingly. The lower limit of detection of the IL-5 assay was 100 pg/ml.

Results

Serum IgE response to Hev b 5

Sera from the latex allergic HCW were tested for IgE reactivity to latex allergens by dot immunoblotting using LAL and rHev b 5/MBP as antigens. MBP was included as a control to exclude the possibility that binding of patient sera to the fusion protein was due to anti-MBP antibodies. All patients except patient 2 demonstrated IgE reactivity to LAL (Fig 2). Sera from patients 1, 2, 3, 4 and 5 showed IgE reactivity to rHev b 5/MBP which was markedly stronger than that to MBP alone indicating the presence of a B cell response to Hev b 5 in these patients. Patient 6 showed IgE reactivity to LAL but not to the rHev b 5/MBP

fusion protein indicating that Hev b 5 was not a significant allergen in this case.

T cell responses to latex allergens

In preliminary primary cultures of PBMC, the optimal response to LAL was observed at 10 or 30 μg/ml in all cases. A representative polyconal response is shown in Fig 3, A). The polyclonal response to rHev b 5/MBP were considerably less than those to LAL. A similar response was observed to MBP alone indicating the presence of MBP-specific T cells in the peripheral blood. Therefore, to enrich for latex specific T cells while preventing the selective expansion of MBP-specific T cells, LAL at 20 μg/ml was used for primary and secondary stimulations. Finally, Hev b 5 specific T cells were enriched by incubating the LAL specific two-week T cell line will rHev b 5/MBP at 20 μg/ml in the third antigen stimulation. The three-week latex specific T cell lines from all six latex allergic donors responded to LAL (Table II). The T cell lines from patients 1 and 2 were also tested for their proliferative responses to rHev b 5/MBP and MBP alone. In contrast to the polyclonal responses, the oligoclonal responses to rHev b 5/MBP were significantly higher than those of MBP alone, indicating a selective expansion of Hev b 5-specific T cells. In fact, MBP responses were only at background levels. Representative responses to LAL, rHev b 5/MBP and MBP are shown (Fig 3, B).

Hev b 5 T cell epitope mapping

The latex specific three-week T cell lines were tested for proliferative responses to the Hev b 5 peptides. T cell reactivity to one or more Hev b 5 peptides was identified in all five donors who demonstrated IgE binding to rHev b 5/MBP (Table II), and a representative T cell proliferative response to the peptide set is shown (Fig 3, B). Patient 6 failed to respond to any peptide. Hev b 5 (46-65) induced T cell proliferation in all five donors with IgE reactivity to rHev b 5/MBP. The stimulation indices observed for these responses were 5.9, 10.8, 5.0, 5.9 and 5.6. Additionally peptide Hev b 5 (109-128) stimulated T cells from three of the five donors.

Production of IL-5 and IFNγ by oligoclonal latex specific T cells

IL-5 was produced by the latex specific T cell line from patient 4 in response to stimulation with selected Hev b 5 peptides that caused T cell proliferation (Fig. 4). Peptides Hev b 5 (37-56), (45-65), (55-74), (64-83) and (109-128) induced T cell proliferation (stimulation indices greater than or equal to 2.5) and also secretion of IL-5 while peptides Hev b (100-119) and (118-137) failed to stimulate either T cell proliferation or cytokine secretion. In contrast secretion of IFNγ by T cells that proliferated in response to Hev b 5 peptides was minimal in all cases (≤100 pg/ml).

Discussion

Latex allergy is a potentially severe, untreatable condition affecting certain high-risk groups including HCW. Hev b 5 is a major latex allergen reacting with serum IgE from 92% of latex allergic HCW. Although the predicted molecular weight of Hev b 5 is 17 kDa this protein migrates at around 26-36 kDa on SDS-PAGE (15, 16). This aberrant migration has been observed for other proteins with are highly acidic and proline rich (16). Although monoclonal antibodies to rHev b 5/MBP have been generated (16), it has been difficult to specifically identify natural Hev b 5 on Wester blots of latex extracts (15, 16, 24). Therefore, we carried out dot-immunoblots using LAL and rHev b 5/MBP as antigen. Dot immunoblot analysis showed LAL-specific and rHev b 5/MBP-specific IgE in sera from patients 1, 3, 4 and 5 indicating the presence of a B cell response to Hev b 5 in these patients. On the other hand patient 6 shoed IgE reactivity to LAL but not to the rHev b 5/MBP fusion protein indicating that Hev b 5 was not a significant allergen in this case. Patient 2 had IgE reactivity to rHev b 5/MBP but not to LAL. A possible explanation for this observation is that the natural Hev b 5 in LAL does not present IgE binding epitopes for this patient's serum. This may be a result of degradation of the neutral Hev b 5 protein cause by the process of ammoniation.

We generated latex specific T cell lines from six latex allergic HCW and analysed the T cell

response to Hev b 5 using overlapping peptides that spanned the entire length of this molecule. The five patients with IgE reactivity to the rHev b 5/MBP by dot blot analysis demonstrated T cell responses to the Hev b 5 peptides. As expected, the latex specific T cell line from patient 6 who lacked IgE reactivity to rHev b 5/MBP failed to proliferate to the Hev b 5 peptides. As T cell lines from five of the six latex allergic patients responded to Hev b 5 peptides we have established that Hev b 5 is a major T cell allergen in the HCW group.

Several peptides of the Hev b 5 molecule stimulated T cell proliferation. Of these peptides, Hev b 5 (46-65) induced proliferation in the T cell lines of all rHev b/MBP IgE-reactive donors while Hev b 5 (109-128) stimulated T cells from 3 of these donors. Both the magnitudes and the frequencies of responses to these peptides suggest they contain immunodominant T cell epitopes of Hev b 5. The high frequency of reactivity is consistent with promiscuous binding of this peptide to different MHC Class II molecules in an outbred human population (25). Such promiscuity is highly desirable for the development of immunotherapeutic approaches in human allergic disease (26).

Several other peptides, Hev b 5 (37-56), Hev b 5 (55-74) and Hev b 5 (118-137) that were overlapping with the 2 immunodominant regions above, stimulated response in one or more subjects (Table II; Fig 5). These may be present shared epitopes or two separate epitopes, and further fine mapping would be required for clarification.

A database search showed Hev b 5 (46-65) to have 60% sequence identity with a peptide sequence of a parasite protein from *Strongyloides stercoralis* which binds IgG and IgE from patients with strongyloidiasis (27). This is of interest as both allergenic and parasitic antigens induce a Th2 polarised cytokine response and induce IgE synthesis. A search for Hev b 5 (109-128) identified 62.5% sequence identity with a region of the kiwi fruit protein pKIWI501. The sequence similarity observed between the whole Hev b 5 and pKIWI501 molecules is most striking in the N and C terminal regions. Therefore, the C-terminal region Hev b 5 (109-128) may contain a T cell epitope that explains the dual reactivity seen clinically with latex and kiwi fruit. All of the five patients in our study with Hev b 5 IgE reactivity

were also known to have food allergy. Interestingly, three of these patients, in addition to latex allergy, described angioedema or anaphylaxis on contact with kiwi fruit. Of the other two patients, one denied kiwi fruit allergy whilst the other patient had avoided kiwi fruit due to fear of possible cross-reactivity. Recognition of antigen components of related allergens (including avocado, banana, kiwi fruit and chestnut) by cross-reactive IgE in latex allergic subjects is well recognised (28) and may lead to multiple allergies. Moreover T cells that recognise cross-reactive epitopes may provide "intermolecular" help (29) and induce the production of specific antibodies to another allergen contributing to multiple clinical allergies.

Murine T cell epitopes of Hev b 5 have been identified previously (30) in BALB/c mice using the same set of overlapping synthetic peptides. Mice were injected with rHev b 5/MBP and subsequent *in vitro* splenocyte proliferation assays were performed to identify T cell determinants. The dominant murine T cell determinants were Hev b 5 (1-20), (37-56), (73-92), (82-101), (109-128), (118-137) and (127-146). We have demonstrated that all seven of these peptides also represent human T cell determinants (Fig 5). Interestingly, the immunodominant human T cell determinant Hev b 5 (46-64) recognised by all 5 subjects with IgE reactivity to rHev b 5/MBP in the current study was only a minor murine T cell determinant (30).

The cytokine data showing marked IL-5 and minimal IFN-γ production by oligoclonal latex specific T cells after stimulation with proliferation inducing peptides are consistent with the immunodominance of the Hev b 5 peptides identified. Peptide specific T cell proliferation and dominant Th_2-type cytokine profile (high IL-5/IFN-γ ratio) are compatible with the T cell response supporting IgE synthesis to this allergen and eosinophil activation which are necessary for the development of clinical allergy.

Abbreviations Used

MBP - maltose binding protein

rHev b 5/MBP - fusion protein of recombinant Hev b 5/MBP

EAST - enzyme allergosorbent test

ELISA - enzyme linked immunosorbent assay

AEU/ml - Allercoat™ EAST units

HCW - health care worker

LAL - low ammoniated latex

PBMC - peripheral blood mononuclear cells

APC - antigen presenting cells

IU - international units

SDS-PAGE - sodium dodecyl sulphate polyacrylamide gel electrophoresis

Table I - Clinical characteristics of latex allergic patients

Patient	Age	Sex	Clinical symptoms on contact with latex	Clinical food allergy	Clinical kiwi fruit allergy	Latex EAST score out of 4 (assay result in AEU/ml)
1	37	F	Urticaria, asthma, angioedema, anaphylaxis	Yes	No	4 (16.4)
2	55	F	Urticaria, angioedema	Yes	Yes	3 (7.1)
3	41	F	Urticaria, rhinitis, asthma, angioedema, anaphylaxis	Yes	Yes	3 (9.5)
4	65	F	Urticaria, rhinitis, asthma, angioedema, anaphylaxis	Yes	Yes	4 (>17.5)
5	30	F	Urticaria, angioedema	Yes	Unknown	4 (>17.5)
6	41	F	Urticaria, rhinitis, asthma, angioedema, anaphylaxis	Yes	Unknown	3 (9.5)

Table II **Proliferative responses to Hev b 5 peptides of latex specific three-week T cell lines from latex allergic patients**

Antigen	P1	P2	P3	P4	P5	P6
p(1-20)	3	2.1	8.2	2.0	**2.6**	2.2
p(10-29)	3.3	1.7	7.7	1.6	1.5	1.7
p(19-38)	2.4	2.4	6.4	1.6	**4.0**	2.0
p(28-47)	7.2	**41.7**	6.9	1.4	1.6	1.1
p(37-56)	8.5	**38.8**	8.8	**3.2**	1.4	1.4
p(46-65)	**21.3**	**22.6**	**25.8**	**5.3**	**5.6**	1.5
p(55-74)	7.7	**5.6**	9.1	**2.8**	1.1	0.9
p(64-83)	5.6	2.1	7.0	**2.6**	2.4	1.0
p(73-92)	2.9	**14.6**	5.3	1.2	0.8	1.3
p(82-101)	5.2	3.2	7.3	**2.6**	1.6	1.2
p(91-110)	2.6	3.2	8.1	2.2	**3.2**	1.2
p(100-119)	2.4	2.2	8.0	1.2	1.0	1.0
p(109-128)	8.2	**42.7**	**17.3**	**3.0**	1.3	0.9
p(118-137)	3.9	**20.8**	8.5	1.2	1.3	0.9
p(127-146)	4.4	**6.6**	8.6	2.2	**2.7**	1.0
p(132-151)	6.5	3.3	7.0	1.7	1.4	1.2
LAL	**9.5**	**12.6**	**65.9**	**3.1**	**4.6**	**27.9**
rHev b 5/MBP	**9.0**	**5.7**	NT	NT	NT	NT
MBP	2.5	1.5	NT	NT	NT	NT
T+APC	3.6	2.1	5.2	0.9	1	1

Latex specific 3-week T cell lines from latex allergic patients (P1-P6) were stimulated with the Hev b 5 peptides at 10 and 30 μg/ml, LAL at 3, 10, 30 and 100 μg/ml and Hev b 5/MBP at 10 μg/ml in 4 day cultures. The maximal response for each peptide and protein antigen is given as counts per minute x 10^{-3} and compared with the control response where T cells and APC were incubated alone in the absence of antigen.
Stimulation indices of ≥ 2.5 are shown in bold type.
NT = not tested

REFERENCES

1. Slater J E; "Latex allergy"; J Allergy Clin Immunol 1994; 94:139-149.

2. Sussman G L *et al*; "The spectrum of IgE-mediated responses to latex"; JAMA 1991; 265:2844-2847.

3. Grzybowski M *et al*; "The prevalence of anti-latex IgE antibodies among registered nurses"; J. Allergy Clin Immunol 1996; 98:535-544.

4. Kibby T and Akl M; "Prevalence of latex sensitization in a hospital employee population"; Ann Allergy Asthma Immunol 1997; 78:41-44.

5. Liss G M *et al*; "Latex allergy: epidemiological study of 1351 hospital workers"; Occ Environ Med 1997; 54:335-342.

6. Brown R H *et al*; "Prevalence of latex allergy among anaesthesiologists: identification of sensitized but asymptomatic individuals"; Anaesthesiology 1998; 89:292-299.

7. Douglas R *et al*; "Prevalence of IgE-mediated allergy to latex in hospital nursing staff"; Aust NZ J Med 1997; 27:165-169.

8. Brugnami G *et al*; "Work-related late asthmatic response induced by latex allergy"; J Allergy Clin Immunol 1995; 96:457-464.

9. Ownby D R *et al*; "Anaphylaxis associated with latex allergy during barium enema examinations"; AJR. 1991; 156:903-908.

10. Chen Z *et al*; "Allergenic and antigenic determinants of latex allergen Hev b 1: peptide mapping of epitopes recognized by human, murine and rabbit antibodies"; Clin Exp Allergy 1996; 26:406-415.

11. Yeang H Y *et al*; "The 14.6 kd rubber elongation factor (Hev b 1) and 24 kd (Hev b 3) rubber particle proteins are recognized by IgE from patients with spina bifida and latex allergy"; J Allergy Clin Immunol 1996; 98:628-639.

12. Sowka S *et al*; "cDNA cloning of the 43-kDa latex allergen Hev b 7 with sequence similarity to patatins and its expression in the yeast Pichia pastoris"; Eur J Bioch 1998; 255:213-219.

13. Scheiner O *et al*; "Cloning and molecular characterization of Hev b 3, a spina-bifida-associated allergen from Hevea brasiliensis latex"; Int Arch Allergy Immunol 1999; 118:311-312.

14. Kostyal D A *et al*; "Cloning and characterization of a latex allergen (Hev b 7): homology to patatin, a plant PLA2"; Clin Exp Immunol 1998; 112:355-362.

15. Akasawa A *et al*; "A novel acidic allergen, Hev b 5, in latex. Purification, cloning and characterization"; J Biol Chem 1996; 271:25389-25393.

16. Slater J E *et al*; "Identification, cloning, and sequence of a major allergen (Hev b 5) from natural rubber latex (Hevea brasiliensis)"; J Biol Chem 1996; 271:25349-24399.

17. Chen Z *et al*; "Identification of hevein (Hev b 6.02) in Hevea latex as a major cross-reacting allergen with avocado fruit in patients with latex allergy"; J Allergy Clin Immunol 1998; 102:476-481.

18. Posch A *et al*; "Latex allergens"; Clin Exp Allergy 1998; 28:134-140.

19. Breitender H; "The Allergens of Hevea brasiliensis"; Allergy Clin Immunol Int 1998; 10:101-109.

20. Brehler R *et al*; "Latex-fruit syndrome - frequency of cross-reacting IgE antibodies"; Allergy 1997; 52:404-410.

21. Raulf-Heimsoth M *et al*; "Analysis of T-cell reactive regions and HLA-DR4 binding motifs on the latex allergen Hev b 1 (rubber elongation factor)"; Clin Exp Allergy 1998; 28:339-348.

22. Suphioglu C *et al*; "Identification of canary grass (Phalaris aquatica) pollen allergens by immunoblotting: IgE and IgG antibody binding studies"; Allergy 1993; 48:273-281.

23. O'Hehir R E *et al*; "Cloned human T lymphocytes as reactive with Dermatophagoides farinae (house dust mite): a comparison of T and B cell antigen recognition"; Immunology 1987; 62:635-640.

24. Beezhold D H *et al*; "Human IgE-binding epitopes of the latex allergen Hev b 5"; J Allergy Clin Immunol 1999; 103:1166-1172.

25. Lake P and Mitchison N A; "Regulatory Mechanisms in the immune response to cell surface antigens"; Cold Spring Harbour Symp Quart Biol 1977; 41:589-593.

26. de Lalla C *et al*; "Identification of novel T cell epitopes in Lol p5a by computational prediction"; J Immunol 1999; 163:1725-1729.

27. Ramachandran S *et al*; "Recombinant cDNA clones for immunodiagnosis of strongyloidiasis"; J Infect Dis 1998; 177:196-203.

28. Moller M *et al*; "Determination and characterization of cross-reacting allergens in latex, avocado, banana, and kiwi fruit"; Allergy 1998; 53:289-296.

29. Hoyne G *et al*; "Characterisation of the specificity and duration of T cell tolerance to intranasally administered peptides in mice: a role for intramolecular epitope suppression"; Int Immunol 1997; 9:1165-1173.

30. Slater J E *et al*; "Murine B-cell and T-cell epitopes of the allergen Hev b 5 from natural rubber latex"; Mol Immunol 1999; 36:135-143.

31. O'Hehir R E *et al*; "House dust mite allergy: from T cell epitopes to immunotherapy"; Eur J Clin Invest 1993; 23:763-772.

32. Schramm G *et al*; "Allergen engineering: Variants of the Timothy Grass Pollen Allergen Phl p 5b with Reduced IgE-binding capacity buy Conserved T Cell reactivity"; J Immunol 1999; 162:2406-2414.

33. Muller U *et al*; "Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom"; J Allergy Clin Immunol 1998; 101:747-754.

34. Sambruck *et al*; "Molecular cloning: a laboratory manual, Second Edition"; Cold Spring Harbour Laboratory Press; Cold Spring Harbour, New York; 1989.

35. Baldari *et al* (1987); Embo J. 6:229-234.

36. Kurjan and Herskowitz (1982); Cell 30:933-943.

37. Schultz *et al* (1987); Gene 54:113-123.

38. Amann *et al*; (1988); Gene 69:301-315.

39. Jameel *et al* (1990); J. Virol. 64:3963-3966.

40. Knapp *et al* (1990); Bio Techniques 8:280-281.

41. Varney *et al*, British Medical Journal, 302:265-269 (1990).

42. Remington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Co, Easton Pennsylvania, USA.

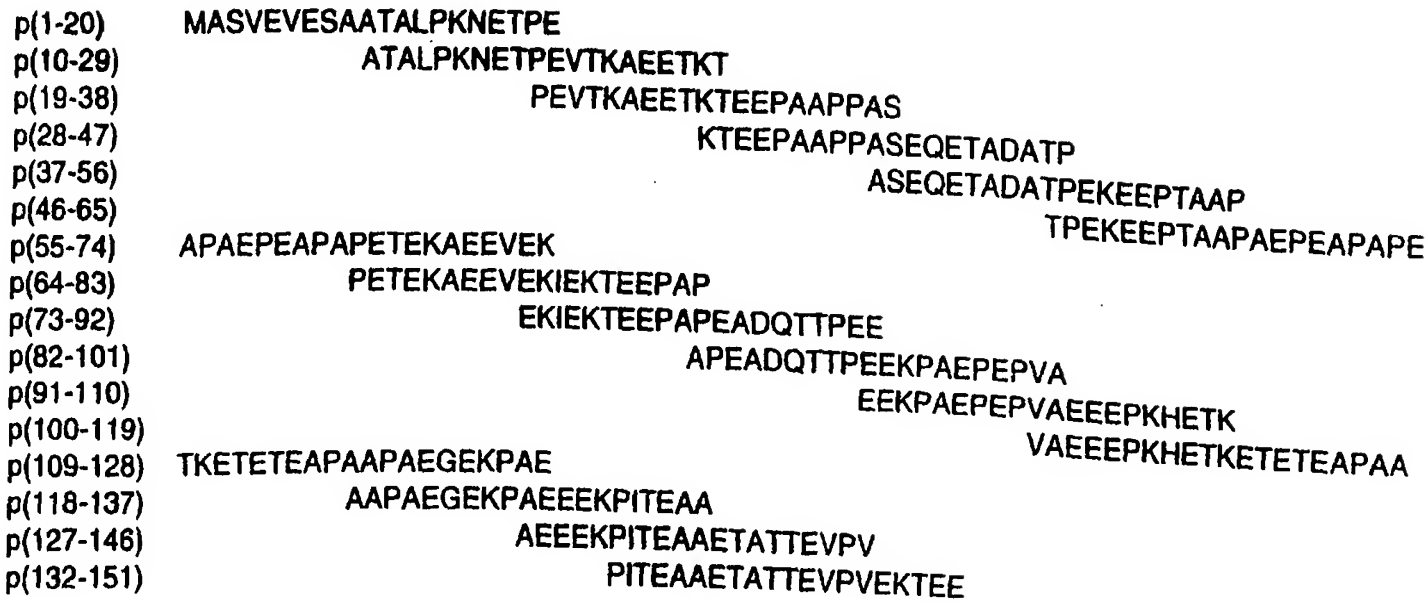
p(1-20) MASVEVESAATALPKNETPE
p(10-29) ATALPKNETPEVTKAEETKT
p(19-38) PEVTKAEETKTEEPAAPPAS
p(28-47) KTEEPAAPPASEQETADATP
p(37-56) ASEQETADATPEKEEPTAAP
p(46-65) TPEKEEPTAAPAEPEAPAPE
p(55-74) APAEPEAPAPETEKAEEVEK
p(64-83) PETEKAEEVEKIEKTEEPAP
p(73-92) EKIEKTEEPAPEADQTTPEE
p(82-101) APEADQTTPEEKPAEPEPVA
p(91-110) EEKPAEPEPVAEEEPKHETK
p(100-119) VAEEEPKHETKETETEAPAA
p(109-128) TKETETEAPAAPAEGEKPAE
p(118-137) AAPAEGEKPAEEEKPITEAA
p(127-146) AEEEKPITEAAETATTEVPV
p(132-151) PITEAAETATTEVPVEKTEE

FIGURE 1

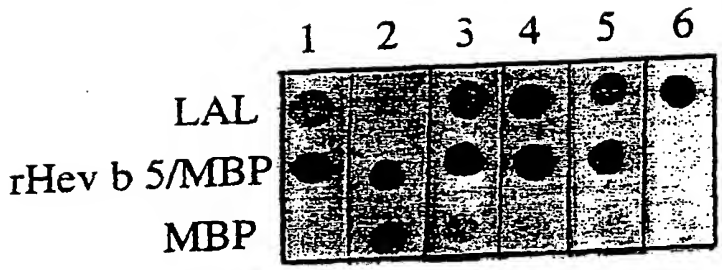

FIGURE 2

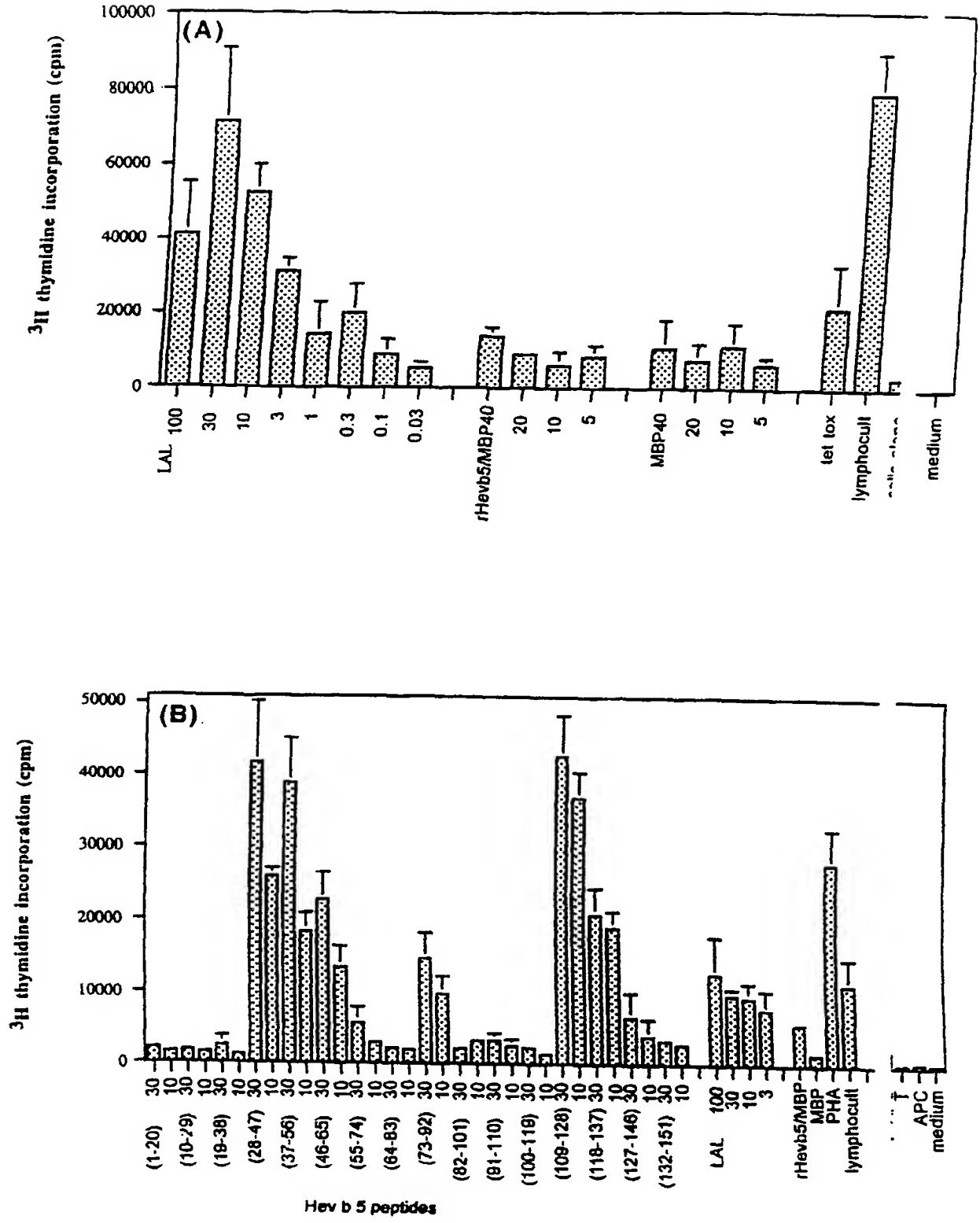
(A)
^{3}H thymidine incorporation (cpm)
100000
80000
60000
40000
20000
0
LAL 100
30
10
3
1
0.3
0.1
0.03
rHevb5/MBP40
20
10
5
MBP40
20
10
5
let lox
lymphocull
medium
(B)
^{3}H thymidine incorporation (cpm)
50000
40000
30000
20000
10000
0
(1-20)
(10-29)
(19-38)
(28-47)
(37-56)
(46-65)
(55-74)
(64-83)
(73-92)
(82-101)
(91-110)
(100-118)
(109-128)
(118-137)
(127-146)
(132-151)
LAL 100
30
10
3
rHevb5/MBP
MBP
PHA
lymphocull
APC
medium
Hev b 5 peptides

FIGURE 3

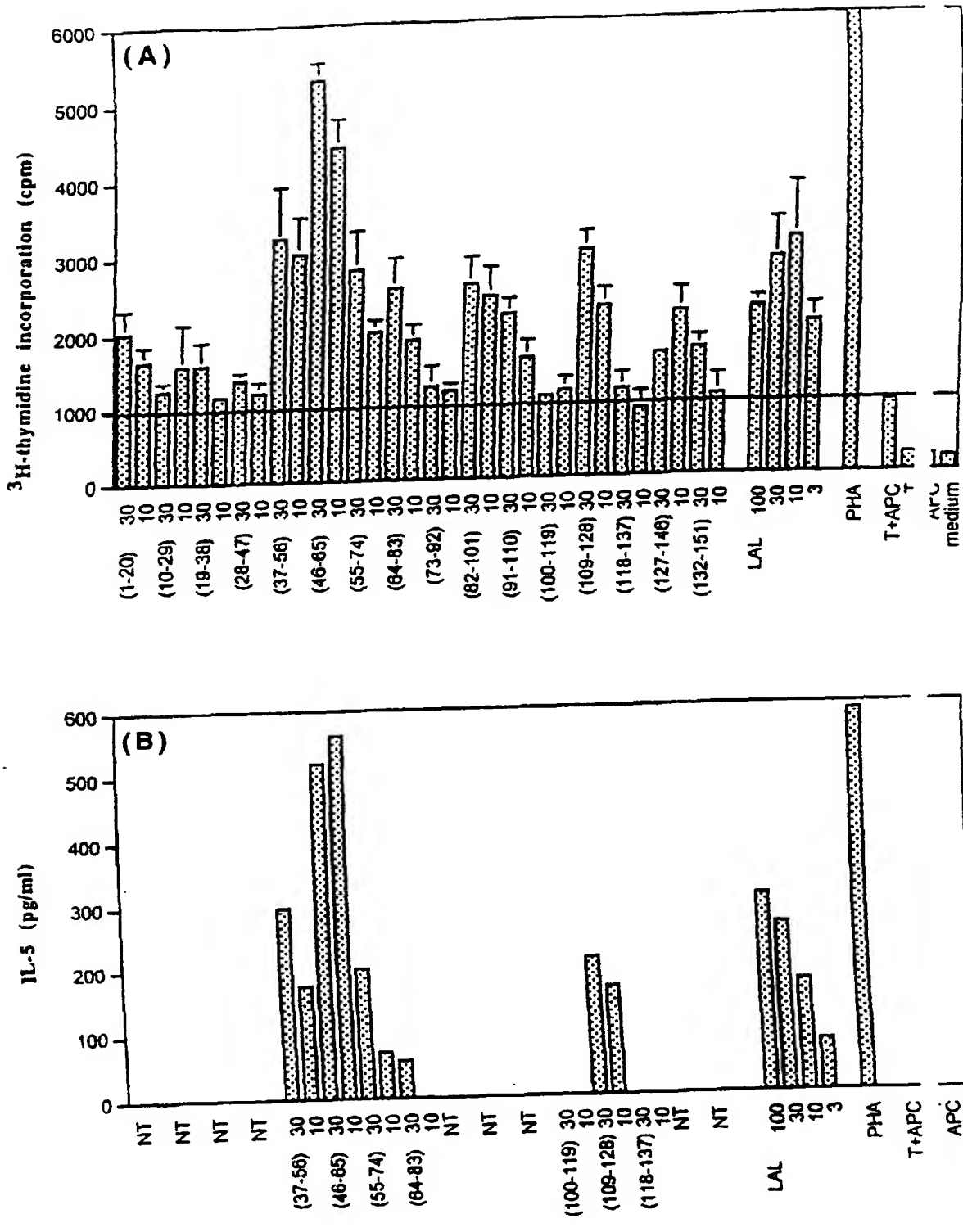
(A)
^{3}H-thymidine incorporation (cpm)
6000
5000
4000
3000
2000
1000
0
(1-20) 30 10
(10-29) 30 10
(19-38) 30 10
(28-47) 30 10
(37-56) 30 10
(46-65) 30 10
(55-74) 30 10
(64-83) 30 10
(73-92) 30 10
(82-101) 30 10
(91-110) 30 10
(100-119) 30 10
(109-128) 30 10
(118-137) 30 10
(127-146) 30 10
(132-151) 30 10
LAL 100 30 10 3
PHA
T+APC
medium
(B)
IL-5 (pg/ml)
600
500
400
300
200
100
0
NT NT NT NT
(37-56) 30 10
(46-65) 30 10
(55-74) 30 10
(64-83) 30 10
NT NT NT
(100-119) 30 10
(109-128) 30 10
(118-137) 30 10
NT NT
LAL 100 30 10 3
PHA
T+APC
APC Medium

FIGURE 4

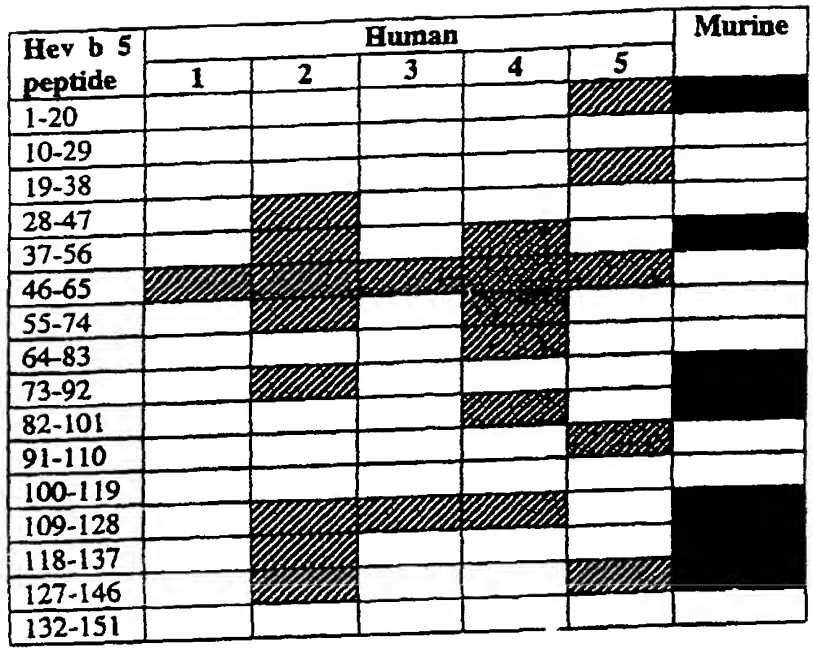
Hev b 5 peptide
Human
1
2
3
4
5
Murine
1-20
10-29
19-38
28-47
37-56
46-65
55-74
64-83
73-92
82-101
91-110
100-119
109-128
118-137
127-146
132-151

FIGURE 5